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Application No.	980668
Date of Filing	12 August, 1998
Applicant	NATIONAL UNIVERSITY OF IRELAND, CORK

PRIORITY DOCUMENT

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Dated this 23rd day of March 1999.

An officer authorised by the
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FORM NO. 1

EP 0980668

REQUEST FOR THE GRANT OF A PATENT

PATENTS ACT, 1992

The Applicant(s) named herein hereby request(s)

[X] the grant of a patent under Part II of the Act

[ ] the grant of a short-term patent under Part III of the Act

on the basis of the information furnished hereunder.

1. Applicant(s)

Name *NATIONAL UNIVERSITY OF IRELAND, CORK*

Address *College Road, Cork, Republic of Ireland*

Description/Nationality *a body Corporate, incorporated by Royal Charter*

2. Title of Invention

"GENETIC DIAGNOSIS OF SUSCEPTIBILITY TO PRE-ECLAMPSIA"

3. Declaration of Priority on basis of previously filed application(s) for same invention (Sections 25 & 26)

Previous filing date	Country in or for which filed	Filing No.
None		

Identification of Inventor(s)

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980668

5. Statement of right to be granted a patent (Section 17(2)(b))

Assignment(s) from the Inventor(s) dated 13 February 1998

6. Items accompanying this Request - tick as appropriate

(i) [X] prescribed filing fee (£ 100)

(ii) [X] specification containing a description and claims
[ ] specification containing a description only
[ ] Drawings referred to in description or claims

(iii) [ ] An abstract

(iv) [ ] Copy of previous application(s) whose priority is claimed

(v) [ ] Translation of previous application whose priority is claimed

(vi) [ ] Authorisation of Agent (this may be given at 8 below if this Request is signed by the Applicant(s))

7. Divisional Application(s)

The following information is applicable to the present application which is made under Section 24 -

Earlier Application No:
Filing Date:

8. Agent

The following is authorised to act as agent in all proceedings connected with the obtaining of a patent to which this request relates and in relation to any patent granted -

Name	Address
TOMKINS & CO.	*5, Dartmouth Road, Dublin 6.*

9. Address for Service (if different from that at 8)

TOMKINS & CO., at their address as recorded for the time being in the Register of Patent Agents.

TOMKINS & CO., Authorised Patent Agents.

Signed Name(s): *by:*
Capacity (if applicant is a body corporate):

Date *12 August 1998*

Genetic diagnosis of susceptibility to pre-eclampsia

Background

The present invention relates to a susceptibility gene for pre-eclampsia and eclampsia, and the use of such a gene in methods for diagnosing susceptibility to these diseases. The invention also relates to a test kit for diagnosis of susceptibility and to pharmaceutical compositions for the prevention or treatment of the diseases. The invention can also be used in the diagnosis of susceptibility to miscarriage and/or miscarriage-related infertility and/or intrauterine growth retardation.

Preeclampsia is the major cause of foetal and maternal morbidity and mortality with probable long term adverse effects on health due to the prolonged associated intrauterine hypoxia. Preeclampsia occurs in approximately five to ten percent of all population births and is uniquely a disease of pregnancy. Acute pathological changes begin to resolve soon after delivery. The pathologic mechanisms causing pre-eclampsia are unclear and no marker predictive for the disease prior to clinical evidence of the disease has been identified. Furthermore an association has been observed between miscarriage and pre-eclampsia (Cooper et al., 1988).

Epidemiological studies show the disease to be highly heritable, mainly confined to first pregnancies and largely prevented by normal first pregnancy by the same partner. Patients affected in first pregnancies have a 7.5% recurrence risk for their second, whereas with a normal first pregnancy, the incidence in the second is of the order of 0.1%. Thus, the first pregnancy appears to have a significant protective effect against pre-eclampsia in a subsequent pregnancy. Therefore, it follows that pre-eclampsia is preventable in principle.

Several classification schemes have been proposed to aid clinical recognition of pre-eclampsia. The classification advocated by the US National Institutes of Health working group on hypertension in pregnancy, is a rise in blood pressure of >15mm Hg diastolic or >30mm Hg systolic from measurement in early pregnancy, or to >140/90 mm Hg in late pregnancy if no early reading is available; plus proteinuria (>0.3g per 24 h) and/or odema. However, in practice, proteinuria measurements may not always be determined and symptoms additional to a rise in blood pressure such as headache, visual disturbance and/or epigastric pain indicate a deterioration in pregnancy consistent with pre-eclampsia and form a basis for clinical intervention of early delivery by caesarian section to resolve the condition.

Although the cause of pre-eclampsia is unknown, hypertension is observed in pre-eclampsia and has been the focus of a large amount of research on the disorder. However, the pathological and physiological changes of pre-eclampsia show that this syndrome is much more than pregnancy-induced hypertension. Evidence to date implicates the action of placental trophoblasts as the underlying cause.

In pre-eclampsia, cytotrophoblast invasion is shallow and spiral arteriol invasion is abnormal, resulting in reduced blood perfusion of the intervillous space. Moreover the

characteristic pattern of integrin switching that takes place during normal trophoblast differentiation does not occur in pre-eclampsia.

The outermost layer (trophoblasts) of the human placenta is devoid of classical class I human leukocyte antigens (HLA-A and HLA-B) and class II proteins (HLA-DR, HLA-DQ and HLA-DP). Although this prevents recognition by maternal T lymphocytes, the lack of class I molecules leaves these cells susceptible to attack by natural killer (NK) cells. However, trophoblast cells directly in contact with maternal tissues selectively express a characteristic nonclassical class Ib molecule, HLA-G. Expression of HLA-G has been shown to be sufficient to protect otherwise susceptible target cells from NK cell mediated lysis. NK cells usually express several different inhibitory receptors of various specificities at the same time. Cross linking of any single inhibitory receptor is sufficient to inactivate NK cell activity against all possible targets. It has been shown that membrane bound HLA-G molecules were able to inhibit alloreactive NK cells with NK inhibitory receptor 1 and inhibitory receptor 2 (NK1 and NK2). It has been shown that CD94 / NKG2 is the predominant inhibitory receptor involved in recognition of HLA-G by decidual and peripheral NK cells. Thus, at a functional level, HLA-G is able to protect target cells from destruction by NK1-,NK2-and NKG2 specific effector cells (Lobe and King, 1997).

More recently, HLA-G has been shown to modulate the ability of blood mononuclear cells to release cytokines (Maejima et al. 1997) suggesting a role for HLA-G in triggering maternal-foetal immune interplay. Specifically, coculturing of HLA-G expressing cells with peripheral blood mononuclear cells (PBMC) increased the amount of interleukin-3 (IL-3) and interleukin-1 beta (IL-1 beta) and decreased the amount of tumour necrosis factor-alpha (TNF-alpha) release from the PBMC cells.

HLA-G binds a diverse array of peptides in a manner similar to that found for classical class I molecules and it has recently been reported that HLA-G is expressed in the human thymus raising the possibility that maternal unresponsiveness to HLA-G expressing foetal tissues may be shaped in the thymus by central presentation of this MHC molecule on the medullary epithelium (Crisa et al. 1997) HLA-G is now known to be capable of stimulating an HLA-G restricted cytotoxic T lymphocyte response and HLA-G molecules can serve as target molecules in lytic reaction with cytotoxic T lymphocytes and HLA-G is involved in education of the lymphocytic repertoire (Schmidt et al., 1997).

Major histocompatibility (MHC) molecules G bind a diverse array of peptides for presentation to T cells as part of mechanism for recognition of self and non-self cells and pathologically altered cells. A detailed analysis of peptides bound to the soluble and membrane HLA-G proteins shows that, like MHC class I molecules, HLA-G also binds a diverse, although less complex array of peptides (Lee et al., 1995). Some of these peptides, which are derived from intracellular proteins, constitute minor histocompatibility antigens which in conjunction with MHC molecules provoke an immune reaction by blood mononuclear cells such as T cells. HLA bound peptides can readily be fractionated, fully or partially purified and sequenced and can be assayed for their capacity to promote an immune reaction by measurement of their capacity to reconstitute lysis of target cells by cytotoxic T cells (den Haan et al., 1998).

The entire gene sequence of HLA-G is known and DNA sequence analysis of HLA-G has shown that the HLA-G gene exhibits limited polymorphism. van der Van & Ober, 1995 examined the first six exons of HLA-G in 45 healthy African-Americans and observed variations in exons 2 and 3, which correspond to the alpha 1 and alpha 2 domains of the peptide binding grove. The most common polymorphism observed was a C to T transition at position 1488, corresponding to codon 93. Another common polymorphism was identified by Harrison +et al, 1993 and is a 14 bp deletion in exon 8 of the gene. These results indicate that HLA-G is a polymorphic gene potentially capable of presenting a wide variety of peptides. Patterns of variability in HLA-G are similar to those of other class I MHC genes, where amino acid substitutions are clustered in the alpha 1 and alpha 2 domains.

Two observations of altered expression of HLA-G in pre-eclampsia have been reported. Colbern et al., 1994 showed that the level of HLA-G in placental tissue was reduced in pre-eclampsia and that the decreased expression appeared to be related to a reduced number of trophoblasts in preeclamptic placental tissue. Hara et al., 1996, showed that clusters of extravillous trophoblasts were devoid of HLA-G in preeclamptic patients.

Inheritance

Several bodies of evidence show that pre-eclampsia and eclampsia are largely under genetic control. However the genetic mechanisms underlying susceptibility to pre-eclampsia remain unclear. This is largely due to confounding factors peculiar to its inheritance. First, the condition is specific to pregnancy and genetic studies to date have not been able to clarify whether the genes responsible are acting through the maternal or foetal genotype or through some interaction between the two. Secondly, pre-eclampsia is largely confined to primagravidas with a much lower incidence in subsequent pregnancies and thirdly, as the condition is specific to pregnancy, the genetic contribution of males is difficult to assess.

Diagnosis of true pre-eclampsia can be complicated by other hypertensive disorders such as essential hypertension and hypertension arising from renal disease. Such hypertensive disorders are distinct from true pre-eclampsia but nonetheless can confound diagnosis and thus pose problems for genetic studies.

The classification of pre-eclampsia by some investigators as a disease of immune dysfunction has prompted a number of studies on the role of the major histocompatibility complex in the genetics of pre-eclampsia.

Kilpatrick et al. (1989) reported an association between susceptibility to pre-eclampsia within families and HLA-DR4. In this study, the frequency of HLA-DR4 was higher in sisters with pregnancy induced hypertension than in sisters with normotensive pregnancies (8/18 [44%] vs 10/54 [19%] and more of them shared HLA-DR4 with their spouses (4/14 [29%] vs 0/29). More recently, Peterson et al. (1994) reported that women with HLA A23/29, B44 and DR7 haplotypes had a significantly increased incidence of both pre-eclampsia and intrauterine growth retardation (IUGR). Of 20

women with the A23/29, B44, DR7 haplotypes, 40% had pre-eclampsia, IUGR or both. However, this was a small study and the A23/29, B44 and DR7 haplotypes ar rare.

At least three studies have further investigated the association between pre-eclampsia and HLA-DR by linkage analysis (Winton et al., 1990; Hayward et al., 1992, Harrison et al., 1997). In these studies, no evidence was found for linkage of the HLA region to pre-eclampsia. Hayward et al. (1992) also investigated several candidate genes and random DNA markers. Overall, no evidence was found for linkage to several candidate genes implicated in the pathogenesis of hypertension and their results excluded linkage to several markers. In these studies, an autosomal recessive model was assumed. Winton et al. (1990) also analysed their data for a HLA-DR-beta RFLP (restriction fragment length polymorphism) using the affected sib pair method and the affected pedigree-member method. Both of these methods make no assumption about the mode of inheritance and neither gave any indication of linkage. The majority of pre-eclampsia cases are considered sporadic. A familial pregnancy-induced hypertensive disorder has been described and two loci have been implicated in the familial form of the disorder, namely, a candidate region on chromosome 4 and the eNOS gene region on chromosome 7 (Harrison et al., 1997, Arngrimsson et al., 1997). The epidemiology of PET is consistent with familial pregnancy-induced hypertensive disorder and sporadic PET being distinct entities.

Humphrey et al., 1995, investigated the HLA-G deletion polymorphism for association with pre-eclampsia. Specifically, preeclamptic patients, offspring of preeclamptic mothers, blood relatives of preeclamptic patients, husbands of preeclamptic patients and a normal control group were genotyped for the polymorphism. The was no detectable association between pre-eclampsia in mothers or in offspring of preeclamptic mothers and the HLA-G deletion polymorphisms.

Karhukorpi et al., 1997 investigated HLA-G polymorphisms for association with recurrent spontaneous miscarriage. Specifically, they showed that there was no association between several HLA-G restriction fragment length polymorphisms and recurrent spontaneous miscarriage.

In the largest study of monozygotic twins, pre-eclampsia was reported in five first pregnancies, and all affected mothers were discordant with their twin. A second well documented report on an identical set of twins also showed clear discordance for pre-eclampsia in their first pregnancies. These observations argue against a recessive model and further support a role for the foetal paternal genotype in the disorder. Furthermore, although the subject of some controversy, pre-eclampsia occurs in mothers with mono- and di-zygotic twins arguing against a recessive foetal genotype and in favour of a dominant paternal gene in the foetus.

Some studies have considered the possibility of changing paternity as a contributing factor in the occurrence of pre-eclampsia in multiparae. Most notably, a strong association between pre-eclampsia and changing paternity has been observed.

Much of the work on pre-eclampsia has been based on the hypothesis of a major susceptibility locus in the affected mother and almost all of the genetic studies to date

have focused on linkage or association between the genotype of the mother and pre-eclampsia. In order to test the hypothesis that foetal HLA-G is the candidate gene for the disorder, we have investigated HLA-G genotypes in preeclamptic and control trios and have shown that HLA-G alleles are associated with both normal and pre-eclampsia pregnancy outcome and with recurrent spontaneous abortion.

According to the present invention there is provided a method of diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) establishing all or part of the HLA-G sequence present in a sample from a female and /or male and/or foetus by analysing the nucleic acid from said sample;
b) determining whether one or more of any variants identified in step (a) are indicative of susceptibility to normal pregnancy or pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility by comparative analysis and /or analysis of activity.

Preferably, the HLA-G sequence variants are established by characterising all or part of the DNA sequence of the HLA-G gene and / or closely linked DNA including HLA-A and HLA-E genes by amplifying all or parts HLA-G or closely linked DNAs and identifying the sequence variants present using one or more sequence variation detection methods.

Suitably, one or more copies of all or parts of the HLA-G gene is amplified by any of several amplification approaches such as the polymerase chain reaction (PCR), nucleic acid sequence based amplification (NASBA), self sustained sequence replication (3SR), transcription-mediated amplification (TMA) and strand displacement amplification. Amplification of a target nucleic acid molecule may also be carried out using a the ligase chain reaction (LCR) and a variation of the LCR which employs a short PCR step (PLCR). Suitably, DNA or mRNA is used as the amplification substrate. Suitably, mRNA is converted into DNA using reverse transcriptase. Suitably, the amplified molecules are analysed directly and /or may be cloned into a vector to facilitate analysis. Suitably, DNA sequence variations are detected by any one or more of a variety of gene variation detection methods including DNA sequencing, glycosylase mediated polymorphism detection, restriction fragment length polymorphism analysis, enzymatic or chemical cleavage assays, hybridisation to DNA probe arrays, allele specific oligonucleotide hybridisation assays, allele specific amplification methods such as the amplification refractory method (ARMS), electrophoretic detection of polymorphisms based on migration through a gel matrix, 5' nuclease assay and ligase chain reaction. Suitably, HLA-G variants associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility are identified by gene association studies. Alternatively, HLA-G variants associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility can be identified by analysis of the activity of HLA-G variants. Suitably, analysis of HLA-G activity is

performed by measuring the interaction of one or more of the HLA-G variants or one or more matching variants with blood mononuclear cells and / or measuring the size and level of HLA-G messenger RNA, and/or measuring peptide binding to the HLA-G variant. HLA-G - blood mononuclear cell activity is measured by assessing blood mononuclear cell activation including assessment of one or more of the following; cell proliferation, cytotoxic response, surface marker expression, cytokine production, conjugate formation and target specificity.

The invention also provides a test kit for the diagnosis of susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising:

a) oligonucleotide primers for amplification of all or part of the HLA-G gene;
b) nested oligonucleotide primers for subsequent amplification of all or part of the exons, and/ or introns and / or promotor region and /or RNA of the HLA-G gene;
c) amplification reagents for amplification of genomic DNA and / or RNA segments, selected from a DNA / RNA polymerase, a reverse transcriptase, the deoxyribonucleotides and /or ribonucleotides ATP, CTP, GTP, TTP and UTP, and reaction buffer;
d) reagents for identifying sequence variants in DNA and / or RNA
e) control DNA and /or RNA.

The invention also provides a method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage related infertility comprising the steps of:

a) establishing all or part of the HLA genotype and / or serotype present in a sample from a female and /or male and/or foetus by analysing the nucleic acid and / or protein and / or cells from said sample
b) comparing the HLA genotypes and /or serotypes identified in step (b) with HLA genotypes / serotypes associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

The invention also provides a further method of diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) incubating blood mononuclear cells and /or a subset of such cells with HLA-G and /or a combination of one or more HLA-G variants thereof and /or cells expressing all or part of the HLA-G gene and /or a combination of one or more variants thereof
b) analysing the activity of the blood mononuclear cells and / or the HLA-G and/or cells expressing one or more HLA-G genes.

Preferably, the blood mononuclear cells are obtained as a blood sample and / or tissue sample from the female and /or are obtained through matching the females blood mononuclear cells with blood mononuclear cells from a donor and / or cell line panel. Preferably populations of T cells and / or NK cells are isolated from the blood sample by

density centrifugation and /or immunoselection. Preferably, blood mononuclear cells matching the females blood mononuclear cells are identified from a test panel by matching the HLA type and/or HLA and/or HLA-G genotype of the female with the HLA type and /or HLA and/or HLA-G genotype of blood mononuclear cells. Preferably, HLA-G matching the male and /or female HLA-G is identified from a test panel by matching the HLA-G type and/or HLA-G genotype of the male and /or female with the HLA-G type and /or HLA-G genotype of HLA-G proteins and/or cells expressing one or more HLA-G genes in the test panel. Preferably, such a test panel is assembled by growing cells expressing one or more HLA-G variants. Such cells may be derived from natural tissue such as placenta and / or created artificially by the introduction of one or more vectors bearing HLA-G gene variants which are capable of promoting the expression of the HLA-G gene into a cell and /or by inducing the expression of native HLA-G in cells. Preferably HLA-G protein is used as a crude preparation and /or fully or partially purified from such cells. HLA-G protein may be loaded with binding peptides naturally or artificially.

Preferably, the HLA-G - blood mononuclear cell interaction is measured by assessing blood mononuclear cell activation including assessment of one or more of the following; cell proliferation, cytotoxic response, surface marker expression, cytokine production, conjugate formation and target specificity.

In a further aspect the invention provides a method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) measuring of soluble HLA-G levels in a maternal and/or foetal blood and/or tissue sample;
b) comparing the levels of HLA-G detected in step a) with the levels associated normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

Preferably HLA-G levels are measured by immunoassay using an antibody for specific HLA-G protein.

The present invention also provides a further method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) analysing HLA-G present in a maternal and/or foetal blood and/or tissue sample to identify variants thereof;
b) comparing any variants of HLA-G detected in step a) with variants associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

Preferably HLA-G variants are identified by immunoassay using an antibody for specific HLA-G protein and /or antibody specific for HLA-G protein variants and / or electrophoretic separation methods and / or chromatographic separation methods. Preferred methods for detecting HLA-G protein and variants thereof include, enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and /or polyclonal antibodies.

According to the present invention there is provided a further method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) obtaining a maternal and/or foetal blood and/or tissue sample;
b) measuring of the level of molecules whose concentration changes as a direct and/or indirect
result of HLA-G action;
c) comparing the levels of molecules measured in step b) with the levels associated with normal
pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth
retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

Preferably the molecules of step (b) are selected from IL-1 beta , IL-2, IL-3, IL-4, IL-6, IL-10 and tumour necrosis factor-alpha. Preferably the levels of such molecules are measured by immunoassay using antibodies specific for the molecules.

According to the present invention there is provided a further method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) obtaining a maternal and/or foetal blood and/or tissue sample;
b) measuring of levels of trophoblast specific markers
c) comparing the levels of molecules measured in step b) with the levels associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

Preferably the trophoblast markers are cytokeratins, pregnancy specific glycoprotein 1, human chorionic gonadotrophin and human placental lactogen. Preferably the levels of such molecules are measured by immunoassay using antibodies specific for the molecules.

Preferably, cloning of all or part of one or more HLA-G genes is achieved by amplification of all or part of one or more HLA-G genes and insertion of all or part of the amplified product into a vector capable of expressing the inserted gene. Preferably,

expression of the HLA-G protein from the cloned gene is achieved by introduction of the expression vector into a suitable host such as a bacterium or an eukaryotic cell in cultur . Preferably, the level of activity of the expressed HLA-G protein is measured by a) directly and /or indirectly assaying the interaction of the HLA-G protein and /or cells expressing HLA-G protein with blood mononuclear cells and / or b) detecting one or more molecules whose level is altered as a result of the interaction of the HLA-G protein and /or cells expressing HLA-G protein with blood mononuclear cells.

The invention also provides a method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) obtaining blood mononuclear cells and / or T cell and /or natural killer cell subsets thereof and /or HLA-G and /or HLA-G expressing cells from a female and /or male and /or foetus and / or test panel;
b) measuring the expression level of one or more genes and / or proteins in the HLA-G expressing cells and /or blood mononuclear cells following interaction with HLA-G and / or HLA-G expressing cells;
c) comparing the expression level identified in step (b) with the expression level in the blood mononuclear cells and / or HLA-G expressing cells in normal pregnancy and/ or pre-eclampsia pregnancy and/or intrauterine growth retardation pregnancy and/or miscarriage pregnancy and/or miscarriage-related infertility.

Preferably, the blood mononuclear cells and / or HLA-G expressing cells are obtained as a blood sample and / or tissue sample. Preferably populations of T cells and / or NK cells are isolated from the blood sample by density centrifugation and /or immunoselection. Preferably HLA-G expressing cells are isolated by immunoselection. Preferably, gene expression is measured by any one or combination of several methods including hybridisation between cDNA and /or RNA from the cells and DNA probes and /or RNA probes and /or DNA probe arrays, quantitative amplification approaches such as quantitative (reverse transcriptase - polymerase chain reaction) RT-PCR, 5' nuclease assay, ribonuclease protection assay and S1 nuclease assay.
Preferably, protein expression is measured by any one or combination of several methods including one dimensional and /or two dimensional gel electrophoresis and staining of proteins and / or detection of one or more proteins using, enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays and Western blotting using monoclonal and /or polyclonal antibodies.

In a further aspect the invention provides a method screening for agents which can potentially be used as diagnostic indicators for pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation by:

a) measuring the expression level of one or more genes and / or proteins in HLA-G expressing cells and /or blood mononuclear cells and / or T cell and /or natural killer cell subsets thereof following interaction with HLA-G and / or HLA-G expressing cells;

b) comparing the expression level identified in step (a) with the expression level in HLA-G expressing cells and /or the blood mononuclear cells and / or T cell and /or natural killer cell subsets thereof following interaction with HLA-G and / or HLA-G expressing cells associated with normal pregnancy and/ or pre-eclampsia pregnancy and/or intrauterine growth retardation pregnancy and/or miscarriage pregnancy and/or miscarriage-related infertility.

Preferably, blood mononuclear cells and / or HLA-G expressing cells are obtained from a female and /or male and /or foetus and /or test panel of blood mononuclear cells and / or HLA-G expressing cells.

Preferably, gene expression is measured by any one or combination of several methods including hybridisation between cDNA and /or RNA from the cells and DNA probes and /or RNA probes and /or DNA probe arrays, quantitative amplification approaches such as quantitative (reverse transcriptase - polymerase chain reaction) RT-PCR, 5' nuclease assay, ribonuclease protection assay and S1 nuclease assay. Preferably, protein expression is measured by any one or combination of several methods including one dimensional and /or two dimensional gel electrophoresis and staining of proteins and / or detection of one or more proteins using, enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays and Western blotting using monoclonal and /or polyclonal antibodies.

The invention also relates to a pharmaceutical composition comprising a pharmaceutically effective amount of HLA-G protein and / or cells expressing HLA-G and / or one or more peptides which binds to HLA-G, blood mononuclear cells from a donor and / or test panel known to interact with HLA-G variants, cytokines and any combination thereof including IL-1 beta , IL-2, IL-3, IL-4, IL-6, IL-10 and tumour necrosis factor-alpha and / or inhibitors of cytokines and /or tumour necrosis factor alpha and / or derivatives of cytokines and /or tumour necrosis factor-alpha, optionally with pharmaceutically-acceptable carriers or excipients.

In a further aspect the invention provides a method for screening potential pre-eclampsia and eclampsia and intrauterine growth retardation and miscarriage and miscarriage-related infertility therapeutic agents selected from:

a) a) identifying agents which alter the expression of HLA-G;
b) identifying agents which alter the activity of HLA-G;
c) identifying agents which mimic the action of HLA-G.
d) identifying agents which bind to HLA-G
e) identifying peptides which bind to HLA-G
f) identifying agents which bind to HLA-G receptors
g) identifying expressed genes using DNA probe arrays in a cellular background in HLA-G expressing cells and / or blood mononuclear cells interacting with HLA-G and / or cells expressing HLA-G interacting with blood mononuclear cells

h) identifying expressed proteins using mass spectrometry methods in HLA-G expressing cells and / or blood mononuclear cells interacting with HLA-G and / or cells expressing HLA-G interacting with blood mononuclear cells.

The invention also provides a method for the prevention of pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility selected from:

a) treatment of a female with all or part of a pharmaceutically effective amount of an effective HLA-G protein and /or peptides which bind to HLA-G and / or cells expressing HLA-G.

b) treatment of a female with all or part of a pharmaceutically effective amount of molecules or inhibitors of molecules whose level or activity is directly or indirectly altered by HLA-G action.

c) treatment of a female with all or part of a pharmaceutically effective amount of molecules which inhibit the interaction between HLA-G and one or more of its receptors

d) treatment of a female with all or part of a pharmaceutically effective amount of an agent which alters HLA-G expression.

e) treatment of a female with all or part of a pharmaceutically effective amount of an agent which alters HLA-G related blood mononuclear cell activity.

f) treatment of a female with all or part of a pharmaceutically effective amount of an agent which mimics all or part of HLA-G action.

g) treatment of a female with blood mononuclear cells that recognise foetal and / or self HLA-G

h) treatment of a female with HLA-G protein and / or cells expressing HLA-G.

The invention also provides a method for the prevention of pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility selected from:

a) introduction of one or more variants of the HLA-G gene and /or its receptor into a female and / or male

b) introduction of an inhibitor of expression of the HLA-G gene and /or its receptor into a female and / or male

c) inactivation of one or more variants of the HLA-G gene and /or its receptor in a female and / or male

The invention also provides a method for improving fertility and pregnancy outcome wherein male and / or female partners and / or sperm and / or ova and / or recipients of fertilised eggs and / or zygotes / and / or embryos are selected on the basis of HLA-G so that their genotypes and /or serotypes are associated with normal pregnancy outcomes and / or not associated with pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

Meth ds

Sample c llecti n. Pre-eclamptic patients were identified as primigravidas under the age of 35 who were delivered by caesarean section at, or prior to 36 weeks gestation because of a deterioration in pregnancy indicative of pre-eclampsia. Diagnostic symptoms were a rise in blood pressure of >15mm Hg diastolic or >30mm Hg systolic from measurement in early pregnancy or to>140/90mm Hg in late pregnancy, and one or more of the following: proteinuria, odema, headache, visual disturbance, epigastric pain. Diagnostic symptoms were completely resolved within 3 months after delivery. Blood samples for DNA extraction were collected from pre-eclamptic and matching control trios. In phase one of the collection, offspring samples were collected while parent samples were collected in phase two. As a result the number of offspring samples exceed the number of parent samples collected. The appropriate informed consent was obtained from all subjects. Control mothers were identified as primigravidas with normal delivery and normal blood pressure. Control offspring were the offspring of primigravidas. All individuals in the trios were Irish and Caucasian by origin.

Genotyping. DNA was isolated from blood samples by standard methods. Primers were designed to specifically amplify exon 3 and exon 8 of HLA-G. PCR amplification was carried out in 25µl reactions, each of which contained 100ng genomic DNA, PCR buffer (100mMTris-HCl pH 8.3 (20°C), 500 mM KCl, 15mM $MgCl_2$), 200µM of each dNTP, 300 nM of each primer and 0.5U *Taq* polymerase (Boehringer). Conditions for amplification of exon 3 were 30 cycles at 94°C for 45 s, 61°C for 45 s, 72°C for 60 s using 5'-TACTCCCGAGTCTCCGGGTCTG-3' as the forward primer and 5'-AGGCGCCCCACTGCCCCTGGTAC-3' as the reverse primer. All of the samples were then genotyped for the HLA-G C1488T polymorphism using the recently described glycosylase mediated polymorphism detection (GMPD) method (Vaughan & McCarthy, 1998). Essentially a 319 bp fragment was amplified by semi-nested PCR from exon 3 using the forward primer 5'-GACCGAGGGGGTGGGGCCAGGTTCT-3' and the reverse primer 5'-AGGCGCCCCACTGCCCCTGGTAC-3'. In the semi-nested amplification reaction dTTP was replaced by dUTP. The 3' end of the forward primer was designed so that the first U incorporated downstream of the forward primer was at, or distal to, the 1488 position. Following amplification using P^{32} end labelled forward primer, glycosylase mediated cleavage of the amplified product was performed. Cleavage products were resolved by denaturing gel electrophoresis (20% polyacrylamide) and visualised by autoradiography. The C allele was detected as a 32 n fragment and the T allele as a 28 n fragment. A 14bp insertion/ deletion polymorphism (I/D) occurs in the non-translated region of the HLA-G gene in exon 8 [27]. Conditions for amplification of exon 8 were 30 cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 60 s using5'-TGTGAAACAGCTGCCCTGTGT-3' as the forward primer and5'-AAGGAATGCAGTTCAGCATGA-3' as the reverse primer. The I/D exon 8 polymorphism was genotyped by size separation of the PCR products on a 10% non denaturing polyacrylamide gel and visualised by staining with ethidium bromide.

Allele specific genotyping. In the majority of cases, maternal and paternal C1488T - I/D exon 8 HLA-G haplotypes could be directly assigned. In cases where all members of a trio were heterozygous for either C1488T or I/D polymorphisms, allele specific

amplification was performed in order to assign haplotypes. This was achieved using allele specific primers which allowed selective amplification of the I or D allele. Following allele specific amplification, the C1488T polymorphism was then genotyped using the GMPD assay described above. Conditions for amplification of the I allele were 30cycles at 94°C for 45 s, 64°C for 45 s, 72°C for 60 s using 5'-TACTCCCGAGTCTCCGGGTCTG-3' as the forward primer and 5'-CAAAGGGAACCCATGAACAAATCTTG-3' as the reverse primer. Conditions for amplification of the D allele were 30 cycles at 94°C for 45 s,56°C for 45 s, 72°C for 60 s using 5'-TACTCCCGAGTCTCCGGGTCTG-3' as the forward primer and 5'-GTTCTTGAAGTCACAAACCCACTTG -3' as the reverse primer.

Statistical analysis. Comparisons of allele frequency, genotypic distribution and foeto-maternal allele sharing for the C1488T and I/D exon 8 polymorphisms between the various cohorts of individuals were performed using χ^2 contingency table analysis. Probability values (p) are presented with the numbers of degrees of freedom as a subscript. The χ^2 test of heterozygous excess among offspring was limited to pre-eclampsia families where parents were capable of producing both heterozygous and at least one class of homozygous offspring.

Table 1: Genotype and allele distribution of the HLA-G C/T-93 and I/D-E8 polymorphisms in controi and pre-eclampsia trios.

Controls	n	93 C/C (%)	93 C/T (%)	93 T/T (%)	Frequency C/T	exon 8 I/I (%)	exon 8 I/D(%)	exon 8 D/D (%)	Frequency I / D
Mothers	53	11(20.8)	30(56.6)	12(22.6)	0.49/0.51	10(18.9)	35(66.0)	8(15.1)	0.52/0.48
Fathers	54	22(40.7)	24(44.5)	8(14.8)	0.63/0.37	10(18.5)	31(57.4)	13(24.1)	0.47/0.53
Offspring	84	29(34.5)	36(42.9)	19(22.6)	0.56/0.44	22(26.2)	47(56.0)	15(17.8)	0.54/0.46
PE									
Mothers	72	26(36.1)	33(45.8)	13(18.1)	0.59/0.41	13(18.1)	42(58.3)	17(23.6)	0.47/0.53
Fathers	69	14(20.3)	44(63.8)	11(15.9)	0.52/0.48	17(24.6)	42(60.9)	10(14.5)	0.55/0.45
Offspring	75	15(20.0)	53(70.6)	7(9.4)	0.55/0.45	14(18.7)	48(64.0)	13(17.3)	0.51/0.49

Table 2: Transmitted and non-transmitted HLA-G haplotypes to offspring in control and pre-eclampsia trios.

HLA-G Haplotype	Maternal transmitted haplotype	Maternal non-transmitted haplotype	Paternal transmitted haplotype	Paternal non-transmitted haplotype
		Control Trios		
C-93/I-E8	11 (0.22)	7 (0.15)	12 (0.24)	12 (0.24)
C-93/D-E8	10 (0.20)	21 (0.44)	22(0.44)	18 (0.36)
T-93/D-E8	5(0.1)	11 (0.23)	6(0.12)	7 (0.14)
T-93/I-E8	24 (0.48)	9 (0.18)	10 (0.2)	13 (0.26)
n	50	48	50	50
		PE Trios		
C-93/I-E8	5 (0.08)	11 (0.17)	10 (0.16)	10 (0.16)
C-93/D-E8	35 (0.56)	25 (0.40)	21(0.33)	22 (0.36)
T-93/D-E8	4 (0.06)	5 (0.08)	3(0.05)	9 (0.15)
T-93/I-E8	19 (0.30)	22 (0.35)	29 (0.46)	20 (0.33)
n	63	63	63	61

Table 3: Genotype mating outcomes for the HLA-G polymorphisms in control and pre-eclampsia trios.

				Control Trios		PE Trios	
Mother	Father	Offspring	mt/mnt/pt/pnt	93***	Exon 8****	93***	Exon 8****
AA	AA	AA	AAAA	4	3	4	1
aa	aa	aa	aaaa	2	0	0	0
AA	aa	Aa	AAaa	2	3	6	1
aa	AA	Aa	aaAA	4	2	0	6
AA	Aa	AA	AAAa	4	1	7	5
AA	Aa	Aa	AAaA	1	3	7	4
aa	Aa	aa	aaaA	4	4	4	5
aa	Aa	Aa	aaAa	2	2	9	5
Aa	AA	AA	AaAA	6	4	3	0
Aa	AA	Aa	aAAA	8	0	7	10
Aa	aa	aa	aAaa	3	1	0	4
Aa	aa	Aa	Aaaa	1	9	5	4
Aa	Aa	AA	AaAa	2	8	0	6
Aa	Aa	Aa	AaaA*	0	4	6	3
			aAAa*	3	1	2	5
			(Aa)**	3	4	6	7
Aa	Aa	aa	aAaA	3	4	2	2
total				52	52	68	68

mt/mnt/pt/pnt = maternally transmitted / maternally non-transmitted / paternally transmitted / paternally non-transmitted. * Allele transmitted assigned from haplotype analysis, ** not possible to determine allele transmitted, ***for C/T-93 matings, A = C-93, a = T-93, ****for I/D-E8 matings, A = I-E8, a = D-E8

Table 4. Comparison of pre-eclampsia and Control trios

Allele frequency	Mothers	Fathers	Offspring	
C/T-93	p_1=0.12	p_1=0.09	p_1=0.91	
I/D-E8	p_1=0.47	p_1=0.22	p_1=0.53	
93-E8 haplotype frequency	p_3=0.032	p_3=0.06	p_3=0.034	
Genotype distribution				
C/T-93	p_2=0.17	p_2=0.041	p_2=0.002	
I/D-E8	p_2=0.49	p_2=0.36	p_2=0.49	
	MT	MNT	PT	PNT
C/T-93	p_1=0.022	p_1=0.9	p_1=0.045	p_1=0.43
I/D-E8	p_1=0.0007	p_1=0.045	p_1=0.057	p_1=0.93
93-E8 haplotype	p_3=0.005	p_3=0.15	p_3=0.074	p_3=0.86

Deviation from Hardy-Weinberg equilibrium

	Control Offspring	Control Mothers	Control Fathers	PE Offspring	PE Mothers	PE Fathers
C/T-93	p_1=0.23	p_1=0.34	p_1=0.73	p_1=0.0002	p_1=0.66	p_1=0.02
I/D-E8	p_1=0.25	p_1=0.02	p_1=0.27	p_1=0.015	p_1=0.15	p_1=0.06

mt/mnt/pt/pnt = maternally transmitted / maternally non-transmitted / paternally transmitted/ paternally non-transmitted. Probability values (p) are presented with the numbers of degrees of freedom as a subscript.

Table 5. Comparisons within pre-eclampsia families and within control trios

a) Comparison of transmitted and non-transmitted alleles

		Controls	PEs
Heterozyogte v.homozygote	C/T-93	p_1=0.53	p_1=0.0006
mating outcome	I/D-E8	p_1=0.76	p_1=0.039
Allele transmitted to offspring	C-93 v. T-93 (TDT)	p=0.48	p=0.57
	I-E8 v. D-E8 (TDT)	p=0.049	p=0.91
Maternal transmitted v.	C-93 v. T-93 (TDT)	p=0.117	p=0.55
non-transmitted alleles	I-E8 v. D-E8 (TDT)	p=0.0006	p=0.17
Paternal transmitted v.	C-93 v. T-93 (TDT)	p=0.49	p=0.87
non-transmitted alleles	I-E8 v. D-E8 (TDT)	p=0.57	p=0.24

b) Test of difference between parent of origin

Maternal transmitted v.	C-93 v. T-93	p_1=0.009	p_1=0.106
paternal transmitted alleles	I-E8 v. D-E8	p_1=0.009	p_1=0.007
	93/E8 haplotypes	p_3=0.033	p_3=0.123
Maternal non-transmitted v.	C-93 v. T-93	p_1=0.867	p_1=0.60
paternal non-transmitted alleles	I-E8 v. D-E8	p_1=0.096	p_1=0.722
	93/E8 haplotypes	p_3=0.612	p_3=0.853

Probability values (p) are presented with the numbers of degrees of freedom as a subscript.

Table 6: Transmitted and non-transmitted HLA-G extended genotypes in control and pre-eclampsia trios.

HLA-G Haplotype		Control	PE	HLA-G Haplotype		Control	Control	PE	PE
MT	PT	Offspring	Offspring	T	NT	Mothers	Fathers	Mothers	Fathers
C-I	C-I	4	1	C-I	C-I	2	2	0	2
C-I	C-D	4	3	C-I	C-D	3	3	3	2
C-D	C-I	3	2	C-D	C-I	1	6	9	1
C-I	T-D	2	0	C-I	T-D	4	3	0	5
T-D	C-I	2	3	T-D	C-I	2	3	1	1
C-I	T-I	1	1	C-I	T-I	1	4	2	1
T-I	C-I	3	4	T-I	C-I	2	1	1	7
C-D	C-D	6	8	C-D	C-D	5	11	14	8
C-D	T-D	1	3	C-D	T-D	1	0	2	1
T-D	C-D	0	1	T-D	C-D	0	0	0	0
C-D	T-I	0	22	C-D	T-I	2	5	10	11
T-I	C-D	12	9	T-I	C-D	13	4	8	11
T-D	T-D	2	0	T-D	T-D	2	2	1	0
T-D	T-I	1	0	T-D	T-I	1	1	2	1
T-I	T-D	1	0	T-I	T-D	4	2	2	3
T-I	T-I	8	6	T-I	T-I	5	3	8	7
	n =	50	63			48	50	63	61

MT: Haplotype transmitted from mother to offspring, PT: Haplotype transmitted from father to offspring

T: Haplotype transmitted to offspring, NT: Haplotype non-transmitted to offspring.

Table 7. Relative risk of foetal, maternal and parent of origin effects in a log linear model

Effect	Relative risks			
	Controls		PE	
	C-93	I-E8	C-93	I-E8
1 or 2 alleles in offspring	0.26*	0.55	3.51*	1.7
1 or 2 alleles in mother	1.06	0.76	0.66	0.98
maternal origin	1.21	3.40**	1.12	0.59
paternal origin	4.16**	1.02	0.74	1.11

* significance at the 5% level
* *significance at the 1% level

Table 8 Transmitted and non-transmitted HLA-G extended genotypes in first and second offspring of normal primigravida mothers

First offspring MT / PT	second offspring MT / PT	moth r	fath r
T-I / T-D	T-I / T-D	T-I / C-I	T-D/ C-I
C-D / C-D	C-D / C-D	C-D / C-D	C-D / C-D
C-D / C-D	C-D / C-D	C-D/ T-I	C-D / C-D
T-I/ C-D	T-I/ C-D	T-I/ C-D	C-D / C-D
C-D / C-D	C-D / C-D	C-D / C-D	C-D / C-D
T-D / C-D	T-I/ C-D	T-D / T-I	C-D / C-D
C-D / C-D	T-I/ C-D	C-D/ T-I	C-D / C-D
T-I / T-D	T-I / T-D	T-I / T-D	T-D / C-D
C-D / C-D	T-D / C-D	C-D / T-D	C-D / C-I
C-D / C-D	T-I/ C-D	C-D/ T-I	C-D / C-D
T-D / C-D	T-I / T-D	T-D / T-I	C-D / T-D
C-D / C-D	C-D / C-D	C-D / C-D	C-D / C-D
C-D / C-D	C-D/ T-I	C-D / C-D	C-D/ T-I
C-D / C-D	C-D / C-D	C-D/ T-I	C-D / C-D
T-I / C-I	T-I/ T-I	C-D/ T-I	C-I / T-I
C-I / T-I	T-D / T-I	C-I / T-D	T-I/ T-I
T-D / C-D	C-D / C-D	T-D / C-D	C-D / C-D
C-D / T-D	C-D / C-D	C-D / C-D	T-D / C-D
C-I / C-D	T-I/ C-D	C-I / T-I	C-D / C-D
C-I / T-I	C-D/ T-I	C-I / C-D	T-I/ C-D
C-D / C-I	C-D / C-D	C-D/ T-I	C-I / C-D
C-D / C-D	C-D / C-D	C-D/ T-I	C-D / C-D
C-D / C-I	C-D / C-I	C-D / C-D	C-I / C-D
C-D / T-D	C-D/ T-I	C-D / C-D	T-D / T-I
C-D / C-D	C-I / T-I	C-D / C-I	C-D/ T-I
C-D / C-D	C-D / C-D	C-D / C-D	C-D / C-D
C-D / C-D	C-D / C-D	C-D / T-D	C-D / C-D

MT, transmitted to offspring from mother; PT, transmitted to offspring from father

Table 9 Transmitted and non-transmitted HLA-G extended genotypes in first and second offspring of primigravida pre-eclampsia mothers

pre-eclampsia offspring	second offspring	mother	father
MT / PT	MT / PT		
1 C-D / C-D	C-D / C-D	C-I / C-D	C-D / C-D
2 T-I/ C-D	C-D / C-D	C-D / T-I	C-I / C-D
3 T-I/ C-D	T-I/ C-D	T-I/ T-I	C-D / T-I
4 C-I / C-D	C-D / C-D	C-I / C-D	C-D / T-I
5 C-D / T-I	C-D / T-I	C-D / C-D	C-D / T-I
6 C-D / T-I	C-D / T-I	C-D / T-D	T-I / T-I
7 C-D / T-I	C-D / T-I	C-D / C-D	C-I / T-I
8 T-D / C-I	T-D / C-I	T-D / C-I	C-I / C-I
9 T-I/ C-D	T-I/ C-D	T-I / T-I	C-D / T-I

MT, transmitted to offspring from mother; PT, transmitted to offspring from father

Table 10 HLA-G haplotypes in recurrent spontaneous abortion couples

Recurrent Spontaneous abortion couples	
Female partner HLA-G Haplotype	Male partner HLA-G Haplotype
H	C-D / C-D
C-D / C-D	T-I / T-I
C-I / C-D	H
C-D / C-D	C-D / C-D
C-D / C-D	C-D / C-D
C-D / T-I	C-D / T-I
C-D / T-I	C-D / T-I

Ascertainment f tri s

Primigravida (first pregnancy) pre-eclampsia trios where the mothers suffered severe pre-eclampsia and a matching control group of normal primigravida trios were identified and sampled. To minimise the possibility of misdiagnosis of PE, we applied stringent criteria to ascertainment of samples. Essentially pre-eclampsia cases were identified as primigravidas under the age of 35 who were delivered by caesarean section at, or prior to, 36 weeks gestation because of a deterioration in pregnancy indicative of PE. Diagnostic symptoms were a rise in blood pressure of >15mm Hg diastolic or >30mm Hg systolic from measurement in early pregnancy or to >140/90mm Hg in late pregnancy, and one or more of the following: proteinuria, odema, headache, visual disturbance, epigastric pain. Diagnostic symptoms were completely resolved within 3 months after delivery. A preliminary survey of the sisters of the pre-eclamptic women in this study did not reveal an increased incidence of the condition, indicating that pre-eclampsia in the cohort of mothers investigated here is sporadic.

HLA-G genotyping and statistical analysis

Several alleles in the HLA-G gene have been described. However, only a few of these occur frequently. A silent C to T substitution occurs commonly at codon 93 (C/T-93) in exon 3 of the gene and a insertion / deletion polymorphism occurs in the non-translated region of the gene in exon 8 (I/D-E8). All individuals in both sets of trios were genotyped for both of these polymorphisms (table 1). In order to analyse both C/T-93 and I/D-E8 polymorphisms together, the HLA-G 93-E8 haplotypes transmitted and non-transmitted to offspring were established (table 2). In those cases where both parents of a trio were heterozygous for C/T-93 and/or I/D-E8 polymorphisms, allele specific amplification was performed in order to assign haplotypes. This was achieved using primers which allowed allele specific amplification of a section of the HLA-G gene from the insertion or deletion in exon 8 to a site 5' of codon 93. Following allele specific amplification, the C/T-93 polymorphism was then genotyped using the glycosylase mediated polymorphism detection method (Vaughan & McCarthy, 1998). Using this approach it was possible to assign haplotypes in every complete trio apart from those where mother, father and offspring were heterozygous for both the C/T-93 and I/D-E8 polymorphisms. Haplotypes are presented on the basis of transmission to offspring in table 2. We also determined the C/T-93 and/or I/D-E8 genotype outcomes of matings in both control (52 trios) and pre-eclampsia (68 trios) trios for analysis (table 3). Calculations presented in table 4 and 5 are derived using data presented and/or derived directly from tables 1 to 3. Comparison of allele and haplotype frequencies and genotype distribution for the C/T-93 and I/D-E8 polymorphisms between (table 4) and within (table 5) the control and pre-eclampsia trios was performed using χ^2 contingency table analysis and transmission disequilibrium testing.

Comparison of HLA-G polymorphisms between control and pre-eclampsia trios

HLA-G C/T-93 and I/D-E8 allele frequencies were not significantly different between control and pre-eclampsia trios (table 4). By contrast, the frequency of the 93-E8 haplotypes differed significantly between control and pre-eclampsia mothers (p_3=0.03) and between control and pre-eclampsia offspring (p_3=0.03) while the

difference observed between control and pre-eclampsia fathers was close to significance (p_3=0.06) (Table 4). The genotype distribution of I/D-E8 did not differ between control and pre-eclampsia cases whereas the distribution C/T-93 genotypes differed markedly in pre-eclampsia trios compared to controls, with a highly significant difference being observed between control and pre-eclampsia offspring (p_2=0.002), between pre-eclampsia and control fathers (p_2=0.041), but not between mothers (Table 4). This reflected a significant excess of C/T-93 heterozygotes over Hardy-Weinberg equilibrium expectations in both pre-eclampsia offspring (p_1=0.0002) and pre-eclampsia fathers (p_1 =0.02). A significant excess of C/T-93 heterozygotes over Hardy-Weinberg equilibrium expectations was also observed for I/D-E8 alleles in pre-eclampsia offspring and surprisingly in control mothers (table 4). Comparison of the frequency of maternally and paternally transmitted C/T-93 and I/D-E8 alleles and 93-E8 haplotypes to offspring revealed significant differences between control and pre-eclampsia offspring for maternally transmitted alleles and haplotypes (table 4) while differences in paternal transmission of C/T-93 was significant and differences in I/D-E8 alleles and 93-E8 haplotypes were close to significance (table 4). In particular, these differences reflected an excess of maternally inherited C-93/D-E8 and paternally inherited T-93/I-E8 haplotypes in the pre-eclampsia offspring by comparison with their respective controls (Table 2). A difference was also observed between non-transmitted maternal I/D-E8 alleles (p_1 =0.045) but not between non-transmitted C/T-93 alleles nor 93-E8 haplotypes.

Comparison of HLA-G polymorphisms within control and pre-eclampsia trios
Heterozygote v. homozygote genotype mating outcomes

In an effort to determine the origin of the C/T-93 and I/D-E8 heterozygote excess in pre-eclampsia offspring, we analysed the genotype outcomes of matings in both control and pre-eclampsia trios. As the mother and father are homozygous for the C/T-93 and / or I/D-E8 polymorphisms in several of the control and pre-eclampsia trios, there is only one possible outcome for the offspring genotype (rows 1-4, table 3). However, in the other control and pre-eclampsia trios, the mother and /or the father is heterozygous for the polymorphism. In these cases, 50% of offspring are expected to be heterozygous. Comparison of the observed and expected number of heterozygotes / homozygotes within the control offspring and within the pre-eclampsia offspring indicates that transmission of alleles to the pre-eclampsia offspring, but not to control offspring, is distorted. Of the 58 and 60 pre-eclampsia trios where a homozygous or heterozygous offspring outcome is possible for the C/T-93 and I/D-E8 polymorphisms respectively, 42 (72%) offspring are heterozygote for C/T-93 (p_1=0.0006) while 38 (63.3%) are heterozygote for I/D-E8 (p_1=0.039). Thus the heterozygote excess observed in pre-eclampsia offspring is not simply a consequence of some unusual population structure and is also unlikely to reflect maternal effects alone.

TDT

To investigate these findings further, data was analysed by the TDT (transmission disequilibrium test) method. Only data from those trios in which at least one parent was heterozygous for the C/T-93 and / or I/D-E8 polymorphisms was used (table 3). When the TDT was applied, the frequency of transmission of the C/T-93 alleles to offspring

was not different than expected. However, transmission frequency of the I/D-E8 alleles to control offspring was significantly distorted (X^2_{TDT}, p=0.049). When maternal and paternal transmission frequencies of the C/T-93 and I/D-E8 alleles were analysed independently by TDT, transmission of the maternal I-E8 allele to control offspring was markedly more frequently than expected (X^2_{TDT}, p=0.0006) (table 5) whereas transmission of paternal alleles were not significantly different. This unexpected finding provides evidence for linkage of a HLA-G allele to normal pregnancy outcome in primigravidas and indicates that the effect is largely due to transmission of the maternal allele. Only six out of thirty one control offspring where the mother was heterozygous for I/D-E8 inherited the maternal D-E8 allele. By contrast, the maternal D-E8 allele was transmitted to twenty one out of thirty four pre-eclampsia offspring (table 3). Taken together, these findings indicate a deficit of maternal D-E8 transmission to control offspring and a deficit maternal I-E8 transmission in pre-eclampsia offspring.

Comparison of parent of origin effects

Comparison of the distribution of maternally and paternally transmitted alleles and haplotypes to offspring within each sample group was in agreement with the transmission distortion observed in the TDT. In control offspring a highly significant difference (table 5, p_1=0.009) between maternal and paternal C/T-93 and I/D-E8 allele frequencies reflected a deficit of maternal C-93 and paternal T-93 alleles, and a deficit of maternal D-E8 and paternal I-E8 alleles (table 2). A contrasting difference was observed between maternally and paternally inherited I/D-E8 alleles in the pre-eclampsia offspring (p_1=0.007) indicating a deficit of maternal I-E8 and paternal D-E8 inheritance (table 2, table 5). In addition an deficit of maternal C-93/D-E8 and paternal T-93/I-E8 haplotypes was observed in controls (p_3=0.03) (table 5). Significant differences were not observed for the non-transmitted C/T-93 and I/D-E8 alleles nor 93-E8 haplotypes (table 5). Maternally and paternally transmitted haplotypes to individual offspring are shown in Table 6. Surprisingly, the maternal C-93/D-E8 paternal T-93/I-E8 haplotype combination occurs in twenty two (34%) of the pre-eclampsia offspring but does not occur in the controls. By contrast, the maternal T-93/I-E8 paternal C-93/D-E8 haplotype combination occurs in about the same number of control and pre-eclampsia offspring.

The inference from the statistical analysis give a good indication of foetal, maternal and parent of origin effects of HLA-G for both normal and pre-eclampsia outcome in first pregnancy. However, these effects are not necessarily mutually exclusive and the relationships between the effects may be partially interdependent and difficult to infer. To address this issue, we analysed the data-set in a log linear model as described by Weinberg et al. 1988. We fitted log linear models, allowing for whether the offspring carried one or more copy of an allele, for whether the mother carried one or more copy of an allele, for a maternal origin effect and for a paternal origin effect (Table 7). Since haplotype allowed inference of allele parent of origin in a number of double heterozygote matings, the remaining uninferred allele origins were assigned equally between father and mother. The log linear model analysis indicates that the foetal C-93 allele is significantly over-represented in pre-eclampsia offspring and, independently, under-represented in control offspring. Parent of origin effects are significant within the control trios, indicating a bias towards paternal inheritance of C-93 and maternal inheritance of I-E8.

The results presented here show that genetic screening of foetal DNA should be of value for predictive testing of susceptibility to pre-eclampsia. This may be achieved by characterising foetal nucleic acid isolated from any material containing nucleic acid of foetal origin in the mother such as amniotic fluid, maternal blood or chorionic villus. Furthermore, the results indicate that genetic screening of parents will also be of value for predictive testing of susceptibility to pre-eclampsia.

Analysis of HLA-G haplotype sharing between offspring and mothers was also performed. No significant difference between pre-eclamptic cases and controls was observed for foetal-maternal sharing of HLA-G alleles or for sharing of the paternally transmitted HLA-G allele. There was no significant difference observed for offspring sex between controls and pre-eclampsia cases.

Analysis of maternal and paternal transmitted and non-transmitted HLA-G haplotype in first and second offspring of normal primigravida and primigravida pre-eclampsia mothers shows that the maternal C-93/D-E8 paternal T-93/I-E8 genotype can occur in the second pregnancy in the absence of pre-eclampsia (table 8 and table 9). This shows that exposure of the mother to foetal HLA-G and /or foetal antigens in first pregnancy protects the mother against pre-eclampsia in subsequent pregnancies. This indicates that maternal tolerance for problematic foetal antigens is induced by exposure of the mother to foetal HLA-G and /or foetal antigens. As pre-eclampsia has been reported to be associated with miscarriage, we established the HLA-G haplotypes in seven recurrent spontaneous abortion couples (table 10). In one case, the female partner was homozygous for the C-93/D-E8 haplotype, whereas the male partner was homozygous for the T-93/I-E8 haplotype. This mating combination was only observed in pre-eclampsia couples. Furthermore, any foetus would have the maternal C-93/D-E8 paternal T-93/I-E8 genotype. The female and male partner in two additional couples were heterozygous for the C-93/D-E8 and T-93/I-E8 haplotypes. This mating combination is very rare in control or pre-eclampsia couples and always results in offspring for the T-93/I-E8 haplotypes. Miscarriage may arise if a foetus heterozygous for the C-93/D-E8 and T-93/I-E8 haplotypes is conceived. Taken together, these data indicate that miscarriage arises through a mechanism dependent on HLA-G haplotypes and that both the transmitted and non-transmitted HLA-G allele play a role in this condition.

HLA-G herein refers to any form of HLA-G and / any complex involving HLA-G including different isoforms of HLA-G arising from alternative splicing pathways, combination of different HLA-G isoforms, secreted HLA-G, membrane bound HLA-G HLA-G with peptides bound and HLA-G associated with beta -2-microglobulin. HLA-G protein refers to any crude, partially and /or fully purified form of HLA-G.

The results show that HLA-G polymorphism plays a major role in predisposition to normal, pre-eclampsia and miscarriage outcome in pregnancy and that haplotypic combinations and parent-of-origin effects mediate the influence of HLA-G polymorphism on these outcomes. The results show a strong association between foetal and paternal HLA-G genotypes and PE, and analysis of heterozygote v. homozygote mating outcomes indicate that transmission of HLA-G alleles to the pre-eclampsia offspring, but not to control offspring, is distorted. The results provide evidence for linkage of the

maternal HLA-G I-E8 allele to normal pregnancy outcome in primigravidas and the observed deficit of maternal D-E8 allele and C-93/D-E8 haplotype transmission to control offspring indicates selection for foetuses on the basis of HLA-G genotype in primigravida normal pregnancies. The transmission distortion of the maternal D-E8 allele to the foetus indicates that the effect seen in normal primigravidas is mediated by the maternal allele acting primarily in the foetus. Thus, the maternal HLA-G imparts a protective effect to the foetus which enhances normal pregnancy outcome. This finding indicates that maternal selection of the HLA-G I-E8 and other protective HLA-G alleles occurs in normal pregnancy. By contrast, the maternal D-E8 allele was prevalent in heterozygous pre-eclampsia offspring, indicating that susceptibility to pre-eclampsia partly arises through the lack of a protective maternal HLA-G allele in the foetus. The chi-squared contingency table analysis agreed with the log linear model analysis in that the C-93 allele was over-represented in pre-eclampsia offspring and a bias towards maternal inheritance of I-E8 was present in controls. Furthermore, the log linear model showed that the foetal C-93 allele is under-represented in control offspring with a strong bias towards paternal inheritance of the allele. This indicates that the paternal C-93 allele also imparts a protective or alternatively does not introduce a problematic effect to the foetus which improves the prospect of a normal pregnancy outcome. These results are in good agreement with the findings observed when maternal and paternal haplotype combinations were constructed for individual control and pre-eclampsia offspring where more than one third of the pre-eclampsia cases had a maternal C-93/D-E8 paternal T-93/I-E8 haplotype combination that was absent in the controls. Taken together, the data indicate a strong association between both maternal and paternal HLA-G alleles acting through the foetus and normal pregnancy outcome and indicate that pre-eclampsia arises through the absence of protective maternal and protective or problematic paternal HLA-G alleles in the foetus. Furthermore, considering that there are likely to be several HLA-G alleles with functional differences, and as more than one third of pre-eclampsia cases can be accounted for by a particular maternal / paternal haplotype combination, the results show that the magnitude of the effect of HLA-G in normal and pre-eclampsia pregnancies is large. A protective foetal-maternal HLA-G allele is likley to arise through the transmission of a dominant maternal allele to the foetus which is recognised as self by the maternal immune system. A protective foetal-paternal allele is likely to arise through cross recognition of the paternal allele as self by the maternal immune system. A problematic foetal-paternal allele is likely to arise through cross recognition of the paternal allele as non- self by the maternal immune system.

The results indicate maternal education of the lymphocyte repertoire for maternal HLA-G during and / or prior to pregnancy and for paternal HLA-G during pregnancy. The results also indicate certain paternal HLA-G alleles are compatible with the maternal immune system while others are less compatible/incopatible. Combinations of less compatible/incompatible paternal HLA-G alleles with maternal alleles which do not protect against the paternal alleles are likely to cause susceptibility to pre-eclampsia and miscarriage.

The fact that second offspring of primigravida normal and pre-eclampsia mothers have the maternal C-93/D-E8 paternal T-93/I-E8 genotype in the absence of pre-eclampsia in the second pregnancy is evidence that maternal education for foetal-paternal antigens occurs during the first pregnancy and that this education is mediated by HLA-G.

It is clear from this work that the polymorphisms analysed and / or closely linked polymorphisms in HLA-G or flanking HLA genes contribute directly to enhancing normal pregnancy outcome and to susceptibility to pre-eclampsia and miscarriage. One likely explanation may be that the polymorphisms reported here destabilise HLA-G mRNA and /or alter the splicing pattern and / or glycosylation pattern of HLA-G. At least twelve different haplotypes have been described for the HLA-G gene. Considering the link observed between HLA-G and recurrent spontaneous abortion, it is likely that the combination of HLA-G alleles in the early foetus and / or the combination of the HLA-G alleles in the mother has serious effects on the outcome of implantation in general and is likely to account for cases of unexplained or idiopathic infertility as well as miscarriage. The previously reported link between pre-eclampsia and intra-uterine growth retardation indicates that the latter is also likely to be linked to parent of origins effects of foetal HLA-G alleles and indicate that maternal HLA-G alleles also play a role in the foetal growth outcome.

HLA-G is capable of protecting otherwise susceptible target cells from natural killer cell mediated lysis through its interaction with inhibitory receptors on natural killer cells. HLA-G is also capable of stimulating an HLA-G restricted lymphocyte response, HLA-G molecules can serve as target molecules in lytic reactions with lymphocytes, and HLA-G is involved in education of the lymphocytic repertoire. Thus, pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation is likely to arise through a mechanism involving blood mononuclear cells such as natural killer cells and cytotoxic T lymphocytes whereby interaction between the female mating partner's T cells and foetal antigens is compromised by comparison with normal pregnancy. Thus, compromised interaction leading to the lack of tolerance leads to cell killing. Compromised interaction also can lead to lack of stimulation of cells expressing HLA-G molecules and /or lack of stimulation of cells interacting with cells expressing HLA-G molecules. The fact that the maternal C-93/D-E8 paternal T-93/I-E8 HLA-G genotype can occur in the second pregnancy of a primigravida pre-eclampsia case without pre-eclampsia indicates that education mediated by foetal HLA-G to foetal antigens occurs in the first pregnancy of such mothers which overcomes compromised interactions in second and subsequent pregnancies . The fact that a deficit of maternal C-93/D-E8 genotypes and an excess of T-93/I-E8 genotypes are transmitted to control offspring but not to pre-eclampsia offspring indicates that selection for foetuses that express antigens for which the mother is educated occurs in normal pregnancy. The fact that pre-eclampsia rarely occurs in a second pregnancy when the first pregnancy has been normal indicates that induction of education to foetal antigens mediated by HLA-G also occurs during and prior to the first pregnancy in normal mothers and that pre-eclampsia, miscarriage, miscarriage-related infertility and intra-uterine growth retardation arises from lack of education and /or inadequate induction of education to the foetal antigens in the female mating partner during and/or prior to pregnancy. Lack of and /or compromised induction of education to paternal antigens such as HLA-G in the foetus and / or a defective HLA-G interaction with natural killer cells could lead to lysis of trophoblasts and /or lack of stimulation of trophoblasts leading to reduced trophoblast function and /or lack of stimulation of cells interacting with trophoblasts. Thus, HLA-G linked conditions such as pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation are likely to arise through blood mononuclear cell

mediated killing of accessible foetal tissues such as trophoblasts and / or lack of stimulation of trophoblastic cells because of compromised HLA-G interaction with blood mononuclear cells trophoblasts and / or lack of stimulation of blood mononuclear cells because of compromised HLA-G interaction with trophoblastic cells. Since major histocompatibility (MHC) molecules like HLA-G interact with blood mononuclear cells including cytotoxic T cells and natural killer cells, there is likely to be abnormal interaction between maternal blood mononuclear cells and foetal cells presenting MHC / MHC-antigen complexes and /or MHC / MHC-antigen complexes secreted from foetal cells in pre-eclampsia, miscarriage and intra-uterine growth retardation by comparison with normal pregnancies. Thus, the blood mononuclear cell response and/or the trophoblast response to such an interaction is likely to be abnormal in the HLA-G associated disorders. In particular, the cytokine response produced as a result of such an interaction is likely to be abnormal by comparison with the normal situation.

Thus, diagnosis of susceptibility to pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation and prediction of pregnancy outcomes may be achieved by direct and indirect measurement of the education in the female mating partner to foetal antigens and / or direct and indirect measurement of the interaction between blood mononuclear cells and HLA-G and/or HLA-G expressing cells. Furthermore, direct and indirect measurement of the education in the female mating partner to foetal antigens and /or direct and indirect measurement of the natural killer cell activity in the female mating partner to HLA-G expressing cells and / or direct and indirect measurement of the interaction between blood mononuclear cells and HLA-G and/or HLA-G expressing cells offers a means to monitor the course of pregnancy.

Induction of education to foetal antigens in the female mating partner by treatment with HLA-G and / or peptides known to bind to HLA-G constitutes a therapeutic means for prevention and /or treatment of pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation and any other HLA-G related disorders.

The finding that combinations of HLA-G variants in the foetus are closely associated with pre-eclampsia coupled to the fact that HLA-G interacts with blood mononuclear cells offers a further means to prevent and /or treat pre-eclmapsia miscarriage, miscarriage-related infertility and intrauterine growth retardation by inhibition and /or alteration of the interaction of HLA-G and /or HLA-G variants with blood mononuclear cells. This may be achieved by any one or combination of approaches including treatment with one or more molecules which recognise HLA-G and / or variants of HLA-G and / or one or more HLA-G receptors on blood mononuclear cells, and /or inactivation of the HLA-G gene and /or HLA-G gene variants and /or one or more HLA-G receptors on blood mononuclear cells. For example, this would be achieved by treatment with HLA-G specific and /or HLA-G receptor specific antibodies which interfere with HLA-G - blood mononuclear cell interaction and / or treatment with one or more enzymes which recognise and alter HLA-G and / or HLA-G receptors on blood mononuclear cells and / or treatment with one or more peptides which bind to HLA-G and / or HLA-G receptors on blood mononuclear cells. Alternatively, inhibition of the interaction of one or more HLA-G variants with blood mononuclear cells may be achieved by inactivating the HLA-G gene or HLA-G gene variant and /or one or more

HLA-G receptors on blood mononuclear cells. This may be achieved through the use of one or more gene inactivating approaches such as treatment with one or more nucleic acid antisense and /or ribozyme molecules which inhibit expression of the HLA-G gene and /or HLA-G gene variant and /or one or more HLA-G receptors on blood mononuclear cells. This may also be achieved by inactivating the HLA-G gene in one or both partners of a mating couple somatically or in the germ line through the use of gene therapy approaches whereby inhibitory nucleic acid based molecules such as antisense, and/or ribozyme are introduced into an individual. This may be also be achieved by inactivating the HLA-G gene in one or both partners of a mating couple somatically or in the germ line through the introduction of all or part of a HLA-G gene in such a way that it recombines with the endogenous HLA-G in the cell and inactivates it. Alternatively, the HLA-G gene and /or variants of the HLA-G gene and /or any of it's receptors may be employed in gene therapy methods in order to increase the amount of expression products of such genes in an individual allowing compensation of any deficiency of HLA-G and /or it's receptors in an individual. Thus, alteration of the interaction of HLA-G and /or HLA-G variants with blood mononuclear cells may be achieved by introduction of one or more HLA-G gene variants into somatic cells and / or into the germline of one of both partners of a mating couple or into the fertilised egg or cells arising from the fertilised egg prior to implantation. This is of particular importance for increased fertility for animal breeding purposes. For example, introduction of one or more HLA-G gene variants into the germline of one of both partner of a mating couple or into the fertilised egg or cells arising from the fertilised egg where the HLA-G variant is compatible with the prospective mother offers a means to improve fertility and pregnancy outcome arising from any incompatibility between foetal HLA-G and maternal cells in the mother.

The fact that induction of education to foetal antigens occurs during pregnancy mediated by HLA-G offers a means of inducing education inlcuding tolerance to HLA-G and / or peptides bound to HLA-G in an individual. Thus treatment of an individual with HLA-G and or / peptides known to bind to HLA-G constitutes a means to induce education in an individual to antigens which contribute to autoimmune disease and to antigens which contribute to transplant rejection.

In normal pregnancy, direct and indirect alteration of the level and /or activity of molecules arising from the interaction of HLA-G expressing foetal cells with blood mononuclear cells such as lymphocytes and natural killer cells permit pregnancy to progress properly. In pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation and any other HLA-G related disorders, the alteration of the level and /or activity of molecules arising from the interaction of HLA-G and /or HLA-G expressing foetal cells with blood mononuclear cells such as lymphocytes and natural killer cells is likely to be compromised by comparison with that occurring during normal pregnancy. Thus, mimicry of the alteration of the level and /or activity of one or more molecules arising from the interaction of HLA-G and /or HLA-G expressing foetal cells with blood mononuclear cells in an individual constitutes a therapeutic means for prevention and / or treatment of pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation and any other HLA-G related disorders.

The deficit of maternal C-93/D-E8 genotypes and the excess of T-93/I-E8 genotypes transmitted to control offspring but not to pre-eclampsia offspring implies

The deficit of maternal C-93/D-E8 genotypes and the excess of T-93/I-E8 genotypes transmitted to control offspring but not to pre-eclampsia offspring implies selection of foetuses in normal pregnancy. For fertility purposes, *in vitro* fertilisation and embryo transfer in animals, selection of one or both mating partners, sperm and/or egg donors and/or embryo recipients based on male and / or female HLA-G genotypes and /or serotypes and /or activity associated with a successful normal first pregnancy offers a means to improve fertility and the success rate of *in vitro* fertilisation and embryo transfer in animals and improve pregnancy outcome.

Since HLA-G protects trophoblasts from blood mononuclear cell mediated killing, direct and indirect measurement of measurable substances which originate from trophoblast cell killing should allow diagnosis of susceptibility to pre-eclampsia, miscarriage, intra-uterine growth retardation , and monitoring of pregnancy for normal progress, and progress towards pre-eclampsia, miscarriage and intra-uterine growth retardation in humans and animals. More specifically, the interaction between MHC molecules such as HLA-G and blood mononuclear cells is known to directly and indirectly alter the synthesis and levels of several cytokines. Similarly, trophoblasts are known to synthesise and secrete several cytokines. In particular, the altered regulation of some of these cytokines would be expected to compromise the foetal - maternal immune interaction and could be manifest as pre-eclampsia and/or eclampsia and / or intrauterine growth retardation and/or miscarriage and/or miscarriage-related infertility. For example, the interaction of HLA-G expressing cells with blood mononuclear cells increases the amount of interleukin-3 (IL-3) and interleukin-1 beta (IL-1 beta) and decreases the amount of tumour necrosis factor-alpha (TNF-alpha) release from the blood mononuclear cells. Trophoblasts are known to produce the immunosuppressive cytokine interleukin 10 - a cytokine that potently inhibits alloresponses in mixed lymphocyte reactions. Trophoblasts are also known to produce interleukin 2, a cytokine that both protects the foetus and in involved in activation of maternal killer cells to protect against invading trophoblasts, interleukin 4 and its receptor, which play a role in regulation of umbilical blood flow mediated through the induction of cyclooxygenase-2, indicating a role for interleukin 4 in vascular tone and blood flow modulation during pregnancy, interleukin 6, which is likely to play a role in tissue remodelling associated with placentation.

Since the indications are that pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation arise through a HLA-G mediated mechanism, there are several obvious methods for screening for agents which can potentially be used as diagnostic indicators and therapeutic agents. Screening of gene expression profiles using DNA probe arrays allows identification of genes expressed in HLA-G expressing cells and in blood mononuclear cells and genes whose expression changes as a result of HLA-G interaction with blood mononuclear cells. Comparison of the gene expression profile in HLA-G expressing cells and / or blood mononuclear cells and /or HLA-G expressing cells interacting with blood mononuclear cells and / or in blood mononuclear cells interacting with HLA-G allows identification of agents which can potentially be used as diagnostic indicators and therapeutic agents for pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation.

HLA-G function and HLA-G expression can be measured. Thus screening for agents which alter the expression and /or function and / or which mimic the function of HLA-G provide a method for screening for potential pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation therapeutic agents.

The HLA-G variants associated with pre-eclampsia and miscarriage and normal pregnancy are likely to have one of a small number of consequences:

i) a variant could result in reduced expression of HLA-G which would be reflected as decreased levels of soluble HLA-G in the serum (The HLA-G primary transcript is alternatively spliced to yield several different mRNAs. One of these alternatively spliced forms includes intron 4. The open reading frame in this mRNA continues into intron 4, terminating 21 amino acids after the α3 domain - encoded by exon 4. Thus, the transmembrane region encoded by exon 5 and the cytoplasmic tail of HLA-G is excluded. The resultant protein is hence soluble). Thus measuring of soluble HLA-G levels and comparing these levels with the normal observed levels would allow one to diagnose susceptibility to pre-eclampsia.

ii) the HLA-G variants associated with pre-eclampsia may result in variations in HLA-G protein which in turn could be detected by protein characterisation of soluble HLA-G. Thus characterisation of HLA-G protein in pregnant females would allow one to diagnose susceptibility to pre-eclampsia.

iii) expression of the HLA-G protein leads directly or indirectly to alterations in the levels of certain molecules such as IL-3, IL-1 beta and / or tumour necrosis factor alpha. The HLA-G genotypes associated with pre-eclampsia may result in changed expression of such molecules. Thus measuring of the levels of such molecules and comparing these levels with the normal observed levels would allow one to diagnose susceptibility to pre-eclampsia.

iv) The HLA-G variants associated with pre-eclampsia may result in increased or decreased expression of paternal and /or maternal HLA-G. This in turn would lead to increased lysis of trophoblasts by NK cells and /or cytotoxic T cells. Thus measuring of the levels of trophoblast specific marker and comparing these levels with thenormal observed levels would allow one to diagnose susceptibility to pre-eclampsia.

Pre-eclampsia presents as hypertension in pregnancy and the underlying pathology is related to abnormalities in vascular tone. Since HLA-G abnormalities are directly associated with pre-eclampsia, it follows in principle that HLA-G is likely to be useful for prevention and treatment of hypertension and vascular disorders. In particular, molecules released through the interaction of HLA-G expressing cells and interacting blood mononuclear cells are likely to have therapeutic value for vascular disease.

References

Arngrimsson, R., Hayward, C., Nadaud, S., Baldursdottir, A., Walker, J.J., Liston, W.A.,
Bjarnadottir, R.I., Brock, D.J., Geirsson, R.T., Connor, J.M., Soubrier, F. Evidence for a familial pregnancy-induced hypertension locus in the eNOS-gene region. Am. J. Hum. Genet. 61,354-362 (1997)

Colbern, G.T., Chiang, M.H. & Main, E.K. Expression of the nonclassic histocompatibility antigen HLA-G by pre-eclampsia placenta. Am. J. Obstet. Gynecol. 170:1244-1250 (1994).

Cooper, D.W., Hill, J.A., Chesley, L.C. & Iverson Bryans, C. Genetic control of susceptibility to eclampsia and miscarriage. Br. J. Obstet. Gynaecol., 95:644-653 (1988).

Crisa, L., McMaster, M., Ishii, J., Fisher, S. and Salomon, D. Identification of a thymic epithelial cell subset sharing expression of the class Ib HLA-G molecule with fetal trophoblasts. *J. Exp. Med.* **186**, 289-298 (1997)

Hara, N., Fuji, T. Yamashita, T., Kozuma, S., Okai, T. & Taketani, Y. Altered expression of human leukocyte antigen G (HLA-G) on extravillous trophoblasts in pre-eclampsia: immunohistologicai demonstration with anti-HLA-G specific antibody "87G" and anti-cytokeratin antibody "CAM5.2". Am J Reprod Immunol 36:349-358 (1996).

Harrison, G.A., Humphrey, K.E., Jakobsen, I.B. & Cooper, D.W. A 14bp deletion polymorphism in the HLA-G gene. Hum. Mol. Genet. 2:2200 (1993).

Harrison, G.A., Humphrey, K.E., Jones, N., Badenhop, R., Gua, G., Elakis, G., Kaye, J.A., Turner, R.J., Grehan, M., Wilton, A.N., Brennecke S.P. & Cooper, D.W. A genomewide linkage study of pre-eclampsia/eclampsia reveals evidence for a candidate region of 4q. Am.J.Hum.Genet., 60, 1158-1167 (1997).

Hayward, C., Livingstone, J., Holloway, S., Liston, W.A. & Brock, D.H. An exclusion map for pre-eclampsia: assuming autosomal recessive inheritance. Am. J. Hum. Genet. 50, 749-757 (1992).

Humphrey, K.E., Harrison, G.A., Cooper, D.W., Wilton, A.N., Brennecke S.P. & Trudinger, B.J. HLA-G deletion polymorphism and pre-eclampsia/ eclampsia. Br. J. Obstet. Gynaecol. 102:707-710 (1995).

Karhukorpi, J., Laitinen, T. & Tiilikainen, A.S. HLA-G polymorphism in Finnish couples with recurrent spontaneous miscarriage. Br. J. Obstet. Gynaecol. 104:1212-1214 (1997).

Kilpatrick, D.C., Liston, W.A., Gibson, F. & Livingstone, J. Association between susceptibility to pre-eclampsia within families and HLA-DR4. Lancet, ii, 1063-1065 (1989).

Lee, N., Malacko, A.R., Ishitani, A., Chen, M., Bajorath, J., Marquardt, H. & Geraghty, D.E. The membrane bound and soluble forms of HLA-G bind identical sets of endogenous peptides but differ with respect to TAP association. Immunity, 3, 591-600 (1995)

Loke, Y.W. & King, A. Immunology of human placental implantation: clinical implications of our current understanding. Mol. Med. Today 3(4): 153-159 (1997).

Maejima, M., Fuji, T., Kozuma, S., Okai, T., Shibata, Y. & Taketani, Y. Presence of HLA-G expressing cells modulates the ability of peripheral blood mononuclear cells to release cytokines. Am. J. Reprod. Immunol., 38, 79-82 (1997).

Peterson, R.D.A., Tuck-Muller, C.M., Spinnato, J.A., Peevy, K., Giattina, K. & Hoff, C. An HLA-haplotype associated with pre-eclampsia and intrauterine growth retardation. Amer. J. Reprod. Immunol., 31, 177-179 (1994).

Schmidt, C.M., Garrett, E. & Orr, H.T. Cytotoxic T lymphocyte recognition of HLA-G in mice. Hum Immunol., 55, 127-139 (1997)

Van der Ven, K. & Ober, C., HLA-G Polymorphism in African Americans, J. Immunol., 153, 5628-5633 (1995).

Vaughan, P. & McCarthy, T.V. A novel process for mutation detection using uracil DNA-glycosylase. Nuc. Acids Res., 26, 810-815 (1998).

Weinberg, C.R., Wilcox, A.J. & Lie, R.T. A log-linear approach to case-parent-triad data: assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parentalimprinting. Am J Hum Genet 62, 969-978 (1998).

Winton, A.N., Cooper, D.W., Brennecke, S.P., Bishop, S.M. & Marshall, P. Absence of close linkage between maternal genes for susceptibility to pre-eclampsia and HLA DR-beta. Lancet, 336, 653-657 (1990).

CLAIMS

1. A method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) establishing all or part of the HLA-G sequence present in a sample from a female and /or male and/or foetus by analysing the nucleic acid from said sample;
b) determining whether one or more of any variants identified in step (a) are indicative of susceptibility to normal pregnancy or pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility by comparative analysis and /or analysis of activity.

2. A method as claimed in claim 1 wherein all or part of the HLA-G sequence is amplified.

3. A method as claimed in claim 2 wherein all or part of the HLA-G sequence is amplified by a method or combination of methods selected from the polymerase chain reaction, nucleic acid sequence based amplification, self sustained sequence replication, transcription-mediated amplification, strand displacement amplification. and the ligase chain reaction.

4. A method as claimed in claim 1to 3 wherein all or part of the HLA-G sequence is cloned into a vector.

5. A method as claimed in claim 1to 4 wherein all or part of the DNA sequence is identified by a method or combination of methods selected from DNA sequencing, glycosylase mediated polymorphism detection, restriction fragment length polymorphism analysis, enzymatic or chemical cleavage analysis, hybridisation to DNA and /or RNA probes and /or DNA probe arrays and /or allele specific DNA and /or RNA probes, allele specific amplification analysis, electrophoretic mobility analysis and 5' nuclease assay analysis.

6. A method as claimed in claim 1 to 4 wherein all or part of HLA-G and /or all or part of one or more variants thereof is expressed as a polypeptide in a prokaryotic and / or eukaryotic cell.

7. A method as claimed in claim 1 to 5 wherein HLA-G variants are determined to be indicative of susceptibility to normal pregnancy or pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility by comparing any HLA-G variants identified with HLA-G sequence variants associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

8. A method as claimed in claim 1to 6 wherein any HLA-G variants identified are determined to be indicative of susceptibility to normal pregnancy or pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage

and/or miscarriage-related infertility by expressing and analysing the activity of all or part of one or more HLA-G variant polypeptides and /or any combination thereof.

9. A test kit for the diagnosis of susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising:

a) oligonucleotide primers for amplification of all or part of the HLA-G gene;
b) nested oligonucleotide primers for subsequent amplification of all or part of the exons, and/ or introns and / or promotor region and /or RNA of the HLA-G gene;
c) amplification reagents for amplification of genomic DNA and / or RNA segments, selected from a DNA / RNA polymerase, a reverse transcriptase, the deoxyribonucleotides and /or ribonucleotides ATP, CTP, GTP, TTP and UTP, and reaction buffer;
d) reagents for identifying sequence variants in DNA and / or RNA
e) control DNA and /or RNA.

10. A method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage related infertility comprising the steps of:

a) establishing all or part of the HLA genotype and / or serotype present in a sample from a female and /or male and/or foetus by analysing the nucleic acid and / or protein and / or cells from said sample
b) comparing the HLA genotypes and /or serotypes identified in step (b) with HLA genotypes / serotypes associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

11. A method as claimed in claim 10 wherein all or part of the HLA-A and /or HLA-E genes are analysed in the female and /or male and/or foetus.

12. A method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage related infertility comprising the steps of:

l) incubating blood mononuclear cells and /or a subset of such cells with HLA-G and /or a combination of one or more HLA-G variants thereof and /or cells expressing all or part of the HLA-G gene and /or a combination of one or more variants thereof
m) analysing the activity of the blood mononuclear cells and / or the HLA-G and/or cells expressing one or more HLA-G genes.

13. A method as claimed in claim 8 and claim 12 wherein the activity of HLA-G and /or any combination of variants thereof and / or the blood mononuclear cells and /or a subset of such cells is performed by one or more of the following procedures
(c) measuring the interaction of HLA-G and /or variants thereof with blood mononuclear cells and / or subsets thereof by assessing one or more of the following with respect to HLA-G expressing cells and /or blood mononuclear

cells; cell proliferation, transformation, cytotoxic response, surface marker expression, cytokine production, conjugate formation and target specificity.

(b) measuring the size and / or level of HLA-G and / or HLA-G messenger RNA

(c) measuring the peptide binding capability of HLA-G and /or variants thereof

(d) measuring the binding capability of the HLA-G and /or variants thereof to a HLA-G receptor

(e) measuring one or more molecules whose level is altered as a result of the interaction of the HLA-G and /or variants thereof and /or cells expressing HLA-G with blood mononuclear cells.

14. A method as claimed in claim 12 to 14 wherein fully and / or partially matching blood mononuclear cells and /or subsets thereof and / or HLA-G variants or gene variants thereof and /or cells expressing all or part of the HLA-G gene variants are selected from a test panel by comparing of HLA-G and /or HLA genotypes and / or HLA-G and /or HLA serotypes of the female and /or male and /or foetus with those of the test panel.

15. A method as claimed in claim 12 to 14 wherein the HLA-G is partially or fully purified from a cell expressing the HLA-G from the cloned gene.

16. A method as claimed in claim 12 to 14 wherein the subset of blood mononuclear cells are T cells and /or natural killer cells.

17. A method as claimed in claim 14 wherein blood mononuclear cells and /or subsets thereof and / or HLA-G variants or gene variants and /or cells expressing all or part of the HLA-G gene variants are assembled to form a test panel.

18. A method as claimed in claim 12, 13, 14 and 17 wherein blood mononuclear cells and / or HLA-G and /or cells expressing HLA-G are obtained from and /or matches all of part of those from a female and /or male and /or foetus.

19. A method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) measuring of soluble HLA-G levels in a maternal and/or foetal blood and/or tissue sample;

b) comparing the levels of HLA-G detected in step a) with the levels associated normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

20. A method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) analysing HLA-G present in a maternal and/or foetal blood and/or tissue sample to identify variants thereof;

b) comparing any variants of HLA-G detected in step a) with variants associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

21. A method as claimed in claim 19 and 20 wherein the HLA-G is detected by immunoassay using one or more antibodies specific for HLA-G and /or variants thereof.

22. A method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps:

a) obtaining a maternal and/or foetal blood and/or tissue sample;
b) measuring of the level of molecules whose concentration changes as a direct and/or indirect
result of HLA-G action;
c) comparing the levels of molecules measured in step b) with the levels associated with normal
pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth
retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

23. A method as claimed in claim 22 wherein the molecules of step (b) are selected from IL-1 beta , IL-2, IL-3, IL-4, IL-6, IL-10 and tumour necrosis factor-alpha.

24. A method as claimed in claim 22 and claim 23 wherein the molecules of step (b) are measured by immunoassay using one or more antibodies specific for such molecules.

25. A method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

(a) obtaining a maternal and/or foetal blood and/or tissue sample;
(b) measuring the levels of trophoblast specific markers; and
(c) comparing the levels of molecules measured in step b) with the levels associated with normal pregnancy and/ or susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

26. A method as claimed in claim 25 wherein the trophoblast specific markers are selected from cytokeratins, pregnancy specific glycoprotein 1, human chorionic gonadotrophin and human placental lactogen.

27. A method as claimed in claim 25 and claim 26 wherein the trophoblast specifics are measured by immunoassay using one or more antibodies specific for the markers.

28. A method for diagnosing susceptibility to pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility comprising the steps of:

a) obtaining blood mononuclear cells and / or T cell and /or natural killer cell subsets thereof and /or HLA-G and /or HLA-G expressing cells from a female and /or male and /or foetus and / or test panel;
b) measuring the expression level of one or more genes and / or proteins in the HLA-G expressing cells and /or blood mononuclear cells following interaction with HLA-G and / or HLA-G expressing cells;
c) comparing the expression level identified in step (b) with the expression level in the blood mononuclear cells and / or HLA-G expressing cells in normal pregnancy and/ or pre-eclampsia pregnancy and/or intrauterine growth retardation pregnancy and/or miscarriage pregnancy and/or miscarriage-related infertility.

29. A method as claimed in claim 28 wherein blood mononuclear cells and / or subsets thereof isolated from a female and /or fully or partially matching cells identified from a test panel are incubated with HLA-G and / or cells expressing all or part of one or more HLA-G genes.

30. A method for screening for agents which can potentially be used as diagnostic indicators for pre-eclampsia, miscarriage, miscarriage-related infertility and intrauterine growth retardation by:

a) measuring the expression level of one or more genes and / or proteins in HLA-G expressing cells and /or blood mononuclear cells and / or T cell and /or natural killer cell subsets thereof following interaction with HLA-G and / or HLA-G expressing cells;
b) comparing the expression level identified in step (a) with the expression level in HLA-G expressing cells and /or the blood mononuclear cells and / or T cell and /or natural killer cell subsets thereof following interaction with HLA-G and / or HLA-G expressing cells associated with normal pregnancy and/ or pre-eclampsia pregnancy and/or intrauterine growth retardation pregnancy and/or miscarriage pregnancy and/or miscarriage-related infertility.

31. A method as claimed in claim 12, 13,14, 19, 20, 28, 29 and 30 wherein HLA-G and /or HLA-G expressing cells and /or blood mononuclear cells and subsets thereof are isolated by immunoselection.

33. A method as claimed in claim 28 and 30 wherein, gene expression and / or protein expression is measured by any one or combination of methods selected from; hybridisation between cDNA and /or RNA from the cells and DNA probes and /or RNA probes and /or DNA probe arrays, quantitative amplification methods, reverse transcriptase - polymerase chain reaction (RT-PCR), 5' nuclease assay, ribonuclease protection assay and S1 nuclease assay, one dimensional and /or two dimensional gel electrophoresis and staining of proteins, detection of one or more proteins using, enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric

assays (IRMA) and immunoenzymatic assays (IEMA), sandwich assays and Western blotting using monoclonal and /or polyclonal antibodies.

34. A pharmaceutical composition comprising a pharmaceutically effective amount of HLA-G and / or cells expressing HLA-G and / or one or more peptides which binds to HLA-G, blood mononuclear cells from a donor and / or test panel known to interact with HLA-G variants, cytokines and any combination thereof including IL-1 beta , IL-2, IL-3, IL-4, IL-6, IL-10 and tumour necrosis factor-alpha and / or inhibitors of cytokines and /or tumour necrosis factor alpha and / or derivatives of cytokines and /or tumour necrosis factor-alpha, optionally with pharmaceutically-acceptable carriers or excipients.

35. A method for screening potential pre-eclampsia and eclampsia and intrauterine growth retardation and miscarriage and miscarriage-related infertility therapeutic agents selected from:

a) identifying agents which alter the expression of HLA-G;
b) identifying agents which alter the activity of HLA-G;
c) identifying agents which mimic the action of HLA-G.
d) identifying agents which bind to HLA-G
e) identifying peptides which bind to HLA-G
f) identifying agents which bind to HLA-G receptors
g) identifying expressed genes using DNA probe arrays in a cellular background in HLA-G expressing cells and / or blood mononuclear cells interacting with HLA-G and / or cells expressing HLA-G interacting with blood mononuclear cells
h) identifying expressed proteins using mass spectrometry methods in HLA-G expressing cells and / or blood mononuclear cells interacting with HLA-G and / or cells expressing HLA-G interacting with blood mononuclear cells.

36. A method for the prevention of pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility selected from:

a) treatment of a female with all or part of a pharmaceutically effective amount of an effective
HLA-G and /or peptides which bind to HLA-G and / or cells expressing HLA-G.
b) treatment of a female with all or part of a pharmaceutically effective amount of molecules or inhibitors of molecules whose level or activity is directly or indirectly altered by HLA-G action.
c) treatment of a female with all or part of a pharmaceutically effective amount of molecules which inhibit the interaction between HLA-G and one or more of its receptors
d) treatment of a female with all or part of a pharmaceutically effective amount of an agent which alters HLA-G expression.
e) treatment of a female with all or part of a pharmaceutically effective amount of an agent which alters HLA-G related blood mononuclear cell activity.

f) treatment of a female with all or part of a pharmaceutically effective amount of an agent which mimics all or part of HLA-G action.
g) treatment of a female with blood mononuclear cells that recognise foetal and / or self HLA-G
h) treatment of a female with HLA-G and / or cells expressing HLA-G
i) treatment of a female with one or more antibodies which bind to HLA-G and / or cells expressing HLA-G and / or any receptor for HLA-G.

37. A method for the prevention of pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility selected from:

a) introduction of one or more variants of the HLA-G gene and /or its receptor into a female and / or male
b) introduction of an inhibitor of expression of the HLA-G gene and /or its receptor into a female and / or male
c) inactivation of one or more variants of the HLA-G gene and /or its receptor in a female and / or male.

38. A method as claimed in any of the previous claims wherein fertility and / or pregnancy outcome are improved by selection of male and / or female partners and / or sperm and / or ova and / or recipients of fertilised eggs and / or zygotes / and / or embryos so that their HLA-G and /or HLA genotypes and /or serotypes are indicative of normal pregnancy outcomes and / or not associated with pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

39. A method as claimed in any of the previous claims wherein fertility and / or pregnancy outcome are improved by selection of male and / or female partners and / or sperm and / or ova and / or recipients of fertilised eggs and / or zygotes / and / or embryos so that the activity of their HLA-G and / or blood mononuclear cells interacting with HLA-G are indicative of normal pregnancy outcomes and / or not associated with pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility.

40. A method as claimed in any of claims 1 to 8, 10 to 39 substantially as described herein.

41. A test kit for the diagnosis of susceptibility to normal pregnancy or pre-eclampsia and/or eclampsia and/or intrauterine growth retardation and/or susceptibility to miscarriage and/or miscarriage-related infertility substantially as described herein.

42. A method as claimed in any of the previous claims for monitoring progress of pregnancy.

43. A pharmaceutical composition substantially as described herein.

44. A method for induction of tolerance to a non-self tissue which comprises: administering HLA-G and /or HLA-G loaded with peptides from the non-self tissue and /or HLA-G expressing cells derived from or related to the non-self tissue.

45. A method for the treatment of autoimmune disease which comprises: administering HLA-G and /or HLA-G loaded with peptides from self tissue and / or with specific autoimmune antigen and /or HLA-G expressing cells to an individual.

46. A method for induction of tolerance to a non-self tissue which comprises: introduction of the HLA-G gene into the non-self tissue so that HLA-G is expressed in all or part of the tissue.

47. A method for the treatment of hypertension and / or vascular disease which comprises: administering HLA-G and /or HLA-G expressing cells and /or HLA-G expressing cells and interacting blood mononuclear cells to an individual.

48. A method as claimed in any of claims 44 to 47 substantially as described herein with reference to the Examples.

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